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ORIGINAL ARTICLE

H_2O_2 Improves Quality of *Radix scutellariae* Through Anti-oxidant Effect

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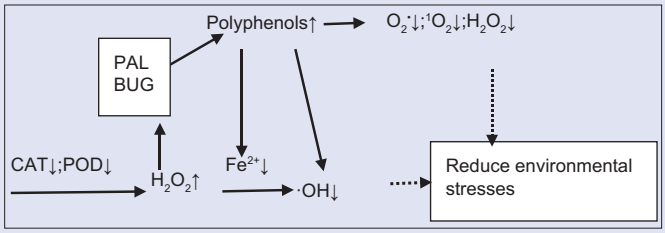
ABSTRACT

Introduction: The correlation between the quality and geographical origin of herbal medicine was traced back to Tang Dynasty in China, more than 1200 years, and the effects of ecological environments on the secondary metabolites such as flavonoids have been confirmed. However, little is known about how the adversity impacts on the quality. Reactive oxygen species (ROS) may be medium between the ecological environment and the secondary metabolism. **Materials and Methods:** The fresh roots of *Scutellaria baicalensis* Georgi were treated with 0.002 μmol/L, 0.2 μmol/L, and 20 μmol/L H_2O_2, respectively. A stress model was established to elucidate the change of secondary metabolism, anti-oxidant enzyme system, and enzymes relating to flavonoids. **Results:** The activities of superoxide dismutase, catalase and peroxidase decreased. Too much H_2O_2, firstly, boosted transformation of flavonoids glycoside into aglucon with the most remarkable activities through UDP-glucuronate baicalein 7-O-glucuronosyltransferase (UBGAT), and β-glucuronidase (GUS), then regulated the gene expression of phenylalanine ammonialyase, GUS, and UBGAT, and increased the contents of flavones, motivated the flavonoid glycoside converting into aglucon. With this action, the flavones displaced the anti-oxidant enzymes. The higher the dosage, the more baicalein and wogonin increased, the later they took action. **Conclusion:** The plant secondary metabolites to keep ROS constant are identical to the effective materials in clinic. They are closely linked. H_2O_2 can improve flavones, especially the aglucon, and further increased the quality of herbal medicine, which possesses very important value in medical practice.

Key words: Anti-oxidase, flavonoid, H_2O_2, *Scutellaria baicalensis*, secondary metabolism

SUMMARY

- H_2O_2 decreasing the activities of CAT and POD lead to accumulation of more H_2O_2. Excess of H_2O_2 up-regulated PAL, BUG, promote biosynthesis of flavones, and enhance the nonenzyme system. "↑" and "↓" represent activity or content "up" and "down" respectively.



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INTRODUCTION

Radix scutellariae is the dried root of the medicinal plant *Scutellaria baicalensis* Georgi, widely used in Eastern and Western medicines. It exhibits a variety of therapeutic effects through flavonoids such as baicalin, baicalein, wogonoside, and wogonin, etc. Now, the synthesis procedure of which has been clarified [Figure 1]. The different varieties of flavonoids play similarly pharmacological roles, but their potential effects vary according to the molecular structures, in descending order of baicalein, baicalin, wogonin, and wogonoside,[1-4] the antibiotic activity of baicalein being 2–5 times, inhibiting IL-1ß coverting enzyme being 1–3 times, more than that of the others. Moreover, baicalein and wogonin are easily absorbed in the stomach and small intestine as well as colon, and the baicalein is 7 times more than the baicalin,[5] these remind us that baicalein and wogonin have an excellent efficient therapy in clinic. Higher baicalein was valued highly, some practices of transforming glycosidase into their aglucon were appreciated,[6,7] therefore, the content of baicalein is major contributor to the quality of *R. scutellariae*, and to the ecological adaptation.

The interrelation between herbal medicine quality and geographical origin even is traced back to Tang Dynasty in China, more than 1200 years, and the effects of ecological environments on the secondary metabolites such as flavonoids have been confirmed.[8-10] The quality of *R. scutellariae* varies according to diverse ecological environments. *S. baicalensis* distributed in China, Japan, Koreas, Russia, Mongolia, etc., that dry climate, and ample sunshine are also the major ecology factors relative to quality of *R. scutellariae* had been confirmed.[11-13] Experience has shown that the optimized producing areas is Cheng-de county (Hebei Province, China), where the baicalein content is higher than other areas, in agreement with ancient experience statement.[14]

The main active constituents in herbal medicine commonly are the secondary metabolites under various abiotic and biotic stresses. The network system to accommodate themselves to the stress is very complicated due to that a metabolic process usually involves a lot of enzymes.[15,16] It had been proved that the modification of a single gene tend to induce unfavorable effects, even disturbs growth and

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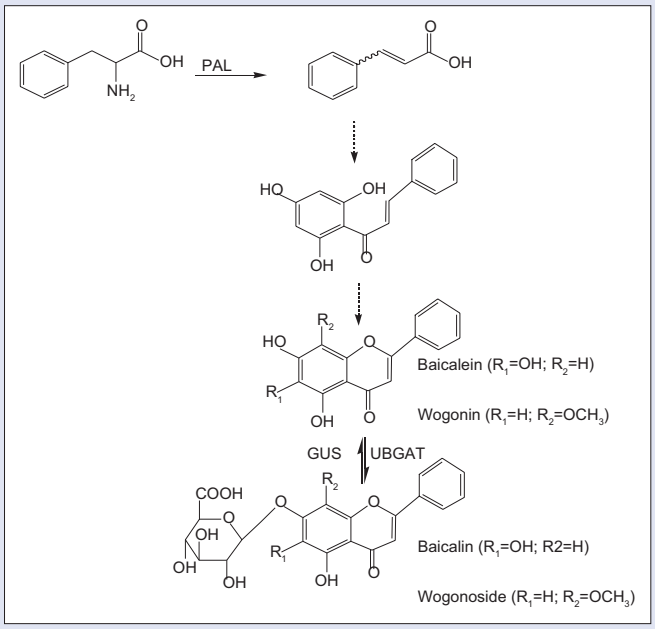

Figure 1: Synthesis and transformations of flavones in *Scutellaria baicalensis*

development.[17] The biological essence of both plant adaption to stress and clinical application is fulfilled by oxhydryl functional group in flavones, the plant secondary metabolites to keep reactive oxygen species (ROS) constant are identical to the effective materials in clinic. They are closely linked, have a cause-and-effect. Stress condition can enable plants to over-production of ROS, while ROS can change metabolism,[18] therefore, ROS may be mediums between the ecological environment and the secondary metabolism, and meddle metabolism of cell to improve the quality of herbal medicine. H_2O_2 is the only ROS that can diffuse through aquaporins in the membranes and over larger distances within cell.[19,20] Due to long-lived molecule compared with other ROS, it can afford to regulate a variety of metabolisms involving in specific biological process, and trigger tolerance against various environmental stresses as the largest contributor to transmit messages.[21-23] With this, a model of adversity mitating essential process of some physiological and biochemical changes in plant may be created to further illuminate how the plants tuned into surroundings. The fresh root of *S. baicalensis* Georgi is a living organism with higher secondary metabolites, the flavones more than 10% in dried root, by which can further clarify the linkage between ecological environment and secondary metabolism, and improve the quality of *R. scutellariae*.

MATERIALS AND METHODS

Experimental

Roots collection and treatment

The 3 years roots of *S. baicalensis* Georgi, were collected and identified by Prof. Meng Xiang-cai, on 10 October 2013 at medicinal botanical garden, Heilongjiang University of Chinese Medicine, China. Some large roots were selected, 1.0 g sample without the xylem was used to determine phenylalanine ammonialyase (PAL), 4.0 g for the β-glucuronidase (GUS) and UDP-glucuronate baicalein 7-O-glucuronosyltransferase (UBGAT).

Some large cylindrical roots were selected, cleaned with water, placed in the 21–23°C shade place for 20 h. Five cylindrical roots were used. Every root was divided into quarters equally, three parts were dipped in 0.002 μmol/L, 0.2 μmol/L, and 20 μmol/L H_2O_2 for 0.5 min respectively, one part was dipped in water. The same disposals were merged into one group, and placed in the shade place (20–25°C). This process repeated 3 times. Above four groups, each was sampled uniformly at the 0, 3, 5, 7, and 9 day, respectively. 0.5 g × 4 g phloem was used for determining the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and polyphenol oxidase (PPO); 0.1 g phloem at the third and the fifth day for analyzing mRNA of PAL, UBGAT, and GUS. The above samples were stored in −80°C refrigerator. Another 3.0 g sample was dried at 60°C for 2 days to determine the contents of baicalin, baicalein, wogonoside, and wogonin. A voucher specimen was deposited at the College of Pharmacy, Heilongjiang University of Chinese Medicine, China.

Determination of enzymes activity

SOD activity (U), assayed based on the reduction of nitroblue tetrazolium (NBT), was defined as the activity of enzyme that caused 50% inhibition of NBT reduction.[24] CAT activity, monitoring the decrease of H_2O_2 at 240 nm for 1 min at 25°C, was calculated as the activity of enzyme that caused a reduction in absorbance at 240 nm of 0.01 per min.[25] POD activity, determined the absorbance changes at 470 nm and 25°C, was defined as the activity of enzyme that caused an increase in absorbance at 470 nm of 0.001 per min.[26] PPO was estimated according to the method of Ohkawa *et al.*[27]

The 1.0 g fresh root was ground in an ice bath with 10 ml 0.1 mol/L boric acid buffer solution (pH 8.8) containing 1.0 mmol/L EDTA, 5% glycerol, 5% polyvinylpyrrolidone; the extracts were centrifuged at − 4°C 10,000 r/min for 20 min. Added above-mentioned supernatant 1.0 ml and 2.0 ml 0.1 mol/L boric acid buffer solution, then added 0.01 ml H_2O, and 0.01 ml 0.4 mol/L H_2O_2 respectively. They were placed in 30°C water, 1 h later, inactivated with 0.2 ml 6 mol/L HCl, centrifuged at 13,000 r/min for 15 min. 0.01 change of OD value, which were measured at 290 nm by the spectrophotometer, was defined as one unit of enzyme activity. PAL activities (U/g . h) = (A290 × extract volume × system volume)/(fresh root weight × enzymes volume × 0.01 × duration).

$U/g \cdot h = U \cdot g^{-1} \cdot h^{-1}$

The 4.0 g fresh root was ground in an ice bath with 40 ml 0.2 mol/L phosphate buffer (pH 6.8) containing 0.1% Vc, 0.02 mol/L catechol, 1.0 mmol/L EDTA, 1 mmol/L PMSF, and 0.6% polyvinylpyrrolidone. The extracts centrifuged at −4°C 10,000 r/min for 20 min. The experiment has three groups, all the groups containing above-mentioned supernatant 1.0 ml. The first group added 0.01 ml H_2O_2 (0.02 mol/L), the second group added 0.01 ml H_2O, the third group contained 0.01 ml H_2O, but 1.0 ml supernatant was boiled. They were placed in 30°C water for 1 h. The reactions were inactivated with 100°C for 15 min, blow-dry with nitrogen, dissolved with 12 ml methanol, centrifuged at 13,000 r/min for 15 min. The joint effect of GUS and UBGAT was expressed with peak area of baicalin measured by HPLC.

Phenylalanine ammonialyase, UDP-glucuronate baicalein 7-O-glucuronosyltransferase and β-glucuronidase expression

Abigen RNA Kit for plant with polysaccharides and polyphenols (ABigen Corporation, Beiking, China) was used for RNA extraction, HiFi-MMLV First Strand cDNA Synthesis Kit (CWbio Co., Ltd., Beiking, China) for bulking of cDNA. The 175 base-pairs fragment of the UBGAT gene of *S. baicalensis* was amplified with the real-time polymerase chain reaction (PCR) method with forward primer-AGCCAAGGAAGCCATAGTCAACG, downstream primer-CCCGAAACAAAGGA AGACGACA; the 131 base-pair of the GUS with forward primer-CAAATACTTTCAT CAATGGTTTCTGGT, downstream

primer- AATGTAGGTGCCGGTTTGGAGTAG; the 139 base-pairs of the PAL with forward primer- TGACCTCGTGCCCCTGTCCTAC, downstream primer- CAGCTCGAAGAACCCTCCACTAACT; the 138 base-pairs of the actin gene with forward primer- TCGACTACGAGCAAGAGCTAGAAACA, downstream primer-TCATTG ATGGCTGGAAGAGGACC; The main reaction mixture was prepared, and include 10 µl 2 × Ultra SYBR mixture, 0.4 µl (10 uM) forward primer, 0.4 µl (10 uM) downstream primer, 2 µl template, and 6.8 µl dH_2O for each reaction (20 µl).

The amplified DNA fragments being the targeted region was confirmed by melting curve analysis. The condition for real-time PCR were 10 min at 95°C and then 15 s at 95°C, 60 s at 60°C; it was performed for 45 cycles. The PCR product was detected by ABI 7500 Applied Biosystems during the reaction using SYBR Green. The relative quantification of gene products is expressed in $2^{-\Delta\Delta CT}$.

Determination of baicalin, baicalein, wogonoside, and wogonin

Waters ACQUITY UPLC with BEH C_{18} (2.1 × 50 mm, 1.7 µm) was used; The gradient mobile phase was consisted of acetonitrile (A) and 0.1% formic acid (B), 25% A; 5–15 min, 25% A → 54% A; 15–22 min, 54% A; 22–23 min, 54% A → 25% A; 23–25 min, 25% A. The mobile phase was delivered at 1 ml/min. The column temperature was 30°C. The peaks of baicalin, baicalein, wogonoside, and wogonin were detected at 277 nm, 279 nm, 274 nm, and 275 nm, respectively. A standard curve of each drug was constructed using weighted linear regression of peak area ratio values of the standards. The percentage of drug recovery after the solid-phase extraction was determined by comparing the internal standard.

The 0.25 g fine powder (d < 0.1 mm) was added to 25 ml volumetric flask, then filled 70% methanol to desired level, ultrasonic extracted for 30 min, made up the loss of methanol. The 1 ml was filtered with 0.22 µm millipore filter.

RESULTS AND DISCUSSION

Effects of H_2O_2 on activities of superoxide dismutase, catalase, peroxidase, and polyphenol oxidase

ROS is considered as the second messenger.[28,29] The moderate concentration of ROS under suitable condition can regulate many responses such as stomatal closure, program cell death, gravitropism, and acquisition of tolerance to both biotic and abiotic stresses,[22] and become an indispensable part of organism lives. Under adversity condition, the levels of ROS are raised significantly,[30] but too much ROS do great harm to the plant cell, even enable it not to survive,[22] usually are quenched by enzymatic components such as SOD, POD, and CAT, etc,[31] and nonenzymatic components such as the Vitamin C, glutathione, polyphenols (flavones etc.), by which remain ROS constant.

The SOD and POD activities showed a modest decrease in fresh *S. baicalensis* roots treated with 0.002 µmol/L, 0.2 µmol/L, and 20 µmol/L H_2O_2, the SOD decreased throughout the process, POD chiefly the fifth day later. The activities of CAT suffered significantly throughout this period, especially, decreased by 71.4–85.7% between the third and fifth day at 0.2 µmol/L, and at 20 µmol/L H_2O_2. CAT and POD are enzymes which catalyze the dismutation of H_2O_2 into water and oxygen. H_2O_2 itself had risen the level of ROS, the fall of CAT and POD activities allowed much more ROS to accumulate still further in the form of H_2O_2. Another study revealed that activity of

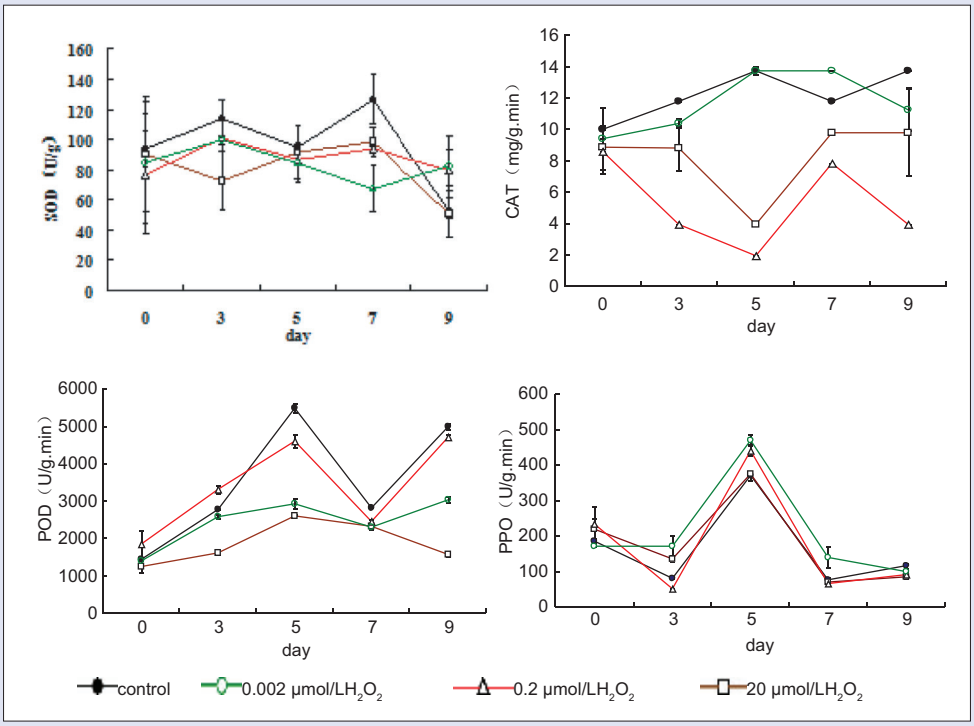

Figure 2: Effects of H_2O_2 on activities of superoxide dismutase, catalase, peroxidase, and polyphenol oxidase. With 0.002 µmol/L, 0.2 µmol/L H_2O_2, and 20 µmol/L H_2O_2, superoxide dismutase, catalase and peroxidase activities suffered, particularly catalase, indicating under stress situation, anti-oxidative enzyme system fails to quench directly excessive reactive oxygen species in the plant cell. The activity of polyphenol oxidase was driven up slightly, showing flavones may be take important role in smoothing reactive oxygen species under heavy stress situation. The performance was repeated 3 times

ascorbate peroxidase with the similar effect of CAT and POD weaken also under drought stress.[32] The fall of CAT and POD activities revealed that anti-oxidative enzyme system undermined the removal of H_2O_2. Although, H_2O_2 has the lowest activity of all ROS,[33] but the accumulation of H_2O_2, without timely scavenged and transformed into OH, would does great harm to cell. In this case, an uneasy relationship between the increase of exogenous H_2O_2 and decline of anti-oxidant enzymes activities made it impossible to deal with the adversity by anti-oxidative enzyme system, a larvate danger still exist. H_2O_2 of different concentrations had a little effect on PPO activities [Figure 2], but with a upward tendency at the 0.002 μmol/L, which may be because at the third day the higher PPO tended to decrease too much flavones produced [Figure 3], and prevent ROS from reducing too much as soon as finite ROS has come near to proper level, while at the 20 μmol/L with

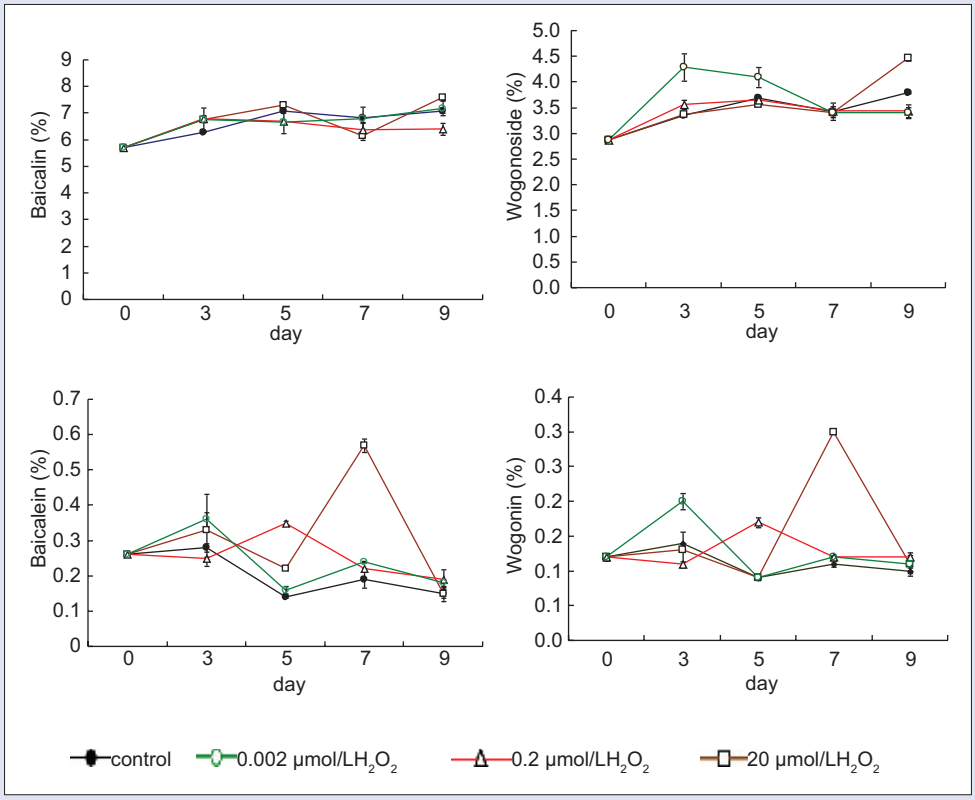

Figure 3: Effects of H_2O_2 on contents of baicalin, baicalein, wogonoside, and wogonin. The contents of both baicalin and wogonoside in roots of *Scutellaria baicalensis* increase soon after treatment with 0.002 μmol/L and 0.2 μmol/L H_2O_2, meanwhile, the contents of both baicalein and wogonin increase, indicating that under slightly stress, flavonoid is the basic and effective tool. At 20 μmol/L H_2O_2, the contents of both baicalein and wogonin increase insignificantly, showing the flavonoid glycoside may be transform into aglucon with higher activity, to work at full strength. Data were based on 3 times repeat

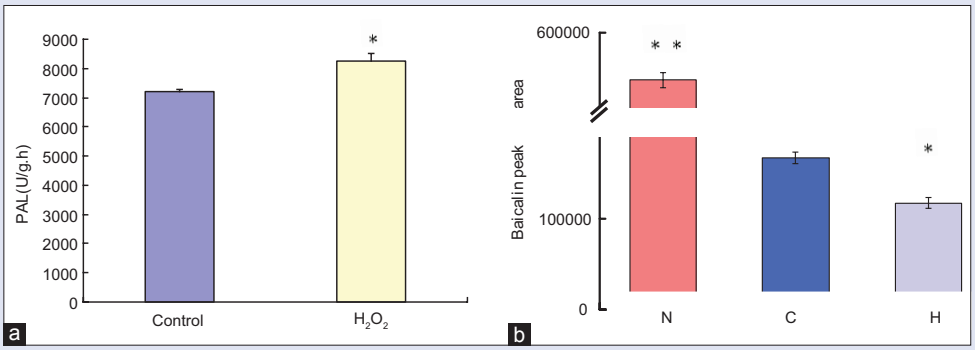

Figure 4: Effects of H_2O_2 on activities of phenylalanine ammonialyase, UDP-glucuronate baicalein 7-O-glucuronosyltransferase, and β-glucuronidase. (a) The effect of H_2O_2 on the phenylalanine ammonialyase, H_2O_2 increasing the activity of phenylalanine ammonialyase significantly. (b) is integrate effect of H_2O_2 on UDP-glucuronate baicalein 7-O-glucuronosyltransferase and β-glucuronidase, "N", "C", and "H" stands for denatured enzymes, control with normal enzyme, treated with H_2O_2 respectively. Compared with denatured enzymes, the baicalin content in the control and the H_2O_2 decreased remarkable, but compared with the control, the H_2O_2 decreased significantly

more ROS, flavones was not enough to smooth too much ROS, and PPO failed to increase, therefore it maintained higher flavones, and further increased anti-oxidant capacity.

Effects of H_2O_2 on contents of baicalin, baicalein, wogpnoside, and wogonin

The PAL, UBGAT, and GUS are enzymes that catalyze synthesis and transformations, the PAL being responsible, indispensable, for flavones synthesis; UBGAT for spare, premier aglucon transformation into flavonoid glycoside; GUS for flavonoid glycoside into aglucon. With ROS, enzymes structure can be modified,[21] which followed by activities change. Structural modification as such made the PAL higher by which contributed to flavones synthesis [Figure 4], and made the GUS higher or made UBGAT lower or both, by which contributed to transformation flavonoid glycoside into aglucon [Figure 4]. Because of both UBGAT and GUS catalyzing transformation between flavonoid glycoside and aglucon, it made impossible to determine the single enzyme to apperceive correlation between glycoside and aglucon. But even so, the fact that baicalin had converted into aglucon is sufficient to reveal how H_2O_2 affects physiological metabolism, and adaptive mechanism dominated by cross-coupling effects of the UBGAT and the GUS. This changes course, without any other steps, would as quickly respond to different circumstance as possible.

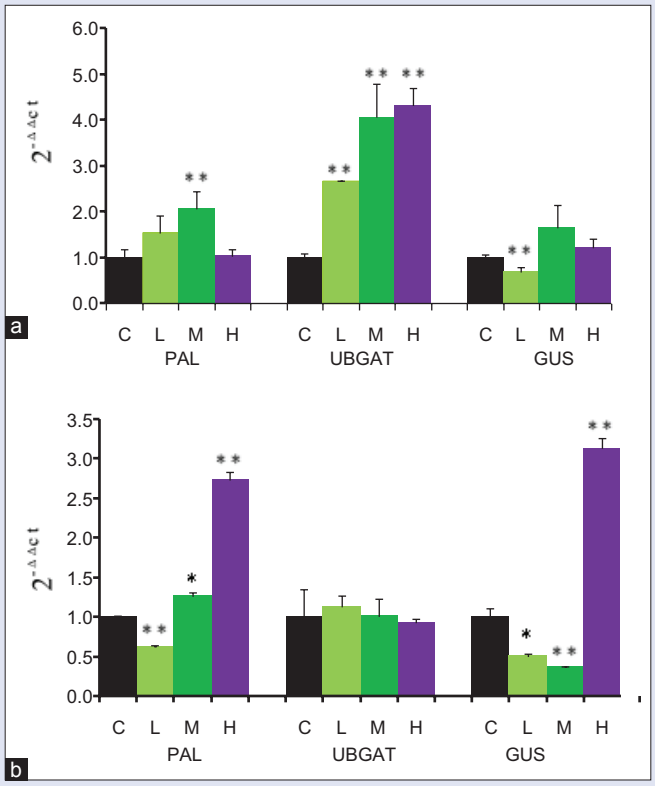

Figure 5: Effects of H_2O_2 on expression of phenylalanine ammonialyase, UDP-glucuronate baicalein 7-O-glucuronosyltransferase, and β-glucuronidase. "C", "L", "M", and "L" stands for control, 0.002 μmol/L, 0.2 μmol/L H_2O_2, and 20 μmol/L H_2O_2 respectively. Ruling the 2-ΔΔct of the control of every gene as 1.0. (a and b) indicate the result of the 3 day and the 5 day respectively. Compared to the control, at the 3 day, 0.002 μmol/L and 0.2 μmol/L H_2O_2 promoted the expression of phenylalanine ammonialyase and UDP-glucuronate baicalein 7-O-glucuronosyltransferase, but phenylalanine ammonialyase with less degree. At the 5 day, 20 μmol/L H_2O_2 increased expression of phenylalanine ammonialyase and β-glucuronidase significantly. *$P < 0.05$, **$P < 0.01$ by

The neatly proportional relationships between the expression of PAL, UBGAT, and GUS and the contents of baicalein, baicalin, wogonin, wogonoside failed to occur, that maybe stem from diverse causes. First of all, usually, the expression of genes is not synchronize with the relevant proteins; moreover, the translation and the posttranslational modifications after the gene transcription can delay the flavonoids rise, for instance, high-expression of PAL at the fifth day but the rise of total flavonoids occur only at the seventh day. Another factor is flavonoids themselves would be destroyed constantly by ROS. More importantly, with artificial intervention, the sudden rise of ROS be surely to disturb the intrinsic metabolic intensively, even in a chaotic state, and a well-balanced state is difficult to be established in such a short time. Therefore, there would be intricate relationships among ROS, genes, and flavonoids. At any rate, the expression of PAL, UBGAT, and GUS are certain to contribute to synthesis and transformation of flavonoids [Table 1]. The flavonoids vary systematically from the 0.002 μmol/L or 0.2 μmol/L to 20 μmol/L H_2O_2.

A lot of evidence support that induction of phenolic metabolism in plants is a response to multiple stresses.[8,34] The plant can produce more phenolics to deal with various environmental stresses. Phenolics, such as flavones, not only can directly scavenge molecular species of active oxygen, chelate transition metal ion Fe^{2+},[35] and reduce Fenton reaction by which H_2O_2 is transformed into the much more OH, a most active ROS with the greatest destructive effect. *S. baicalensis* is particularly rich in flavones, as much as 10% in dried root. Baicalin, baicalein, wogonoside, and wogonin, the major flavones in *S. baicalensis*, have the powerful function of anti-oxidant and scavenging action.[36,37]

H_2O_2 is an important and necessary messenger. It proved that as soon as H_2O_2 was improved, the metabolism would be modified so swiftly that more than 30 kinds of proteins had been expressed in cell within 30 min.[38] 0.002 μmol/L and 0.2 μmol/L H_2O_2 at the third day modulated over-expression of PAL [Figure 5] and improved biosynthesis of all flavonoids [Figure 3] in spite of flavonoids abatement by ROS, baicalin increased from 6.28% to 6.80%, wogonoside from 3.35% to 4.29% at the 0.002 μmol/L. This effect comes near to the natural environment.[11-13] But, at the fifth day, the PAL and UBGAT came back to normal state [Figure 5], which may be due to that finite amount of H_2O_2 can be scavenged by existing amount of enzymes. More flavonoid

Table 1: Effects of different concentration H_2O_2 on flavones (mmol/g)

	Day	Baicalin	Wogonoside	Baicalein	Wogonin	Total flavones
Control	0	1.50	0.63	0.08	0.04	2.25
	3	1.41	0.73	0.10	0.05	2.29
	5	1.59	0.80	0.05	0.03	2.47
	7	1.53	0.75	0.07	0.04	2.39
	9	1.59	0.80	0.06	0.04	2.49
0.002 μmol/L	0	1.50	0.63	0.08	0.04	2.25
	3	1.52	0.93	0.13	0.07	2.65
	5	1.54	0.89	0.06	0.03	2.52
	7	1.52	0.74	0.09	0.04	2.39
	9	1.61	0.74	0.07	0.04	2.46
0.2 μmol/L	0	1.50	0.63	0.08	0.04	2.25
	3	1.52	0.77	0.09	0.04	2.42
	5	1.49	0.79	0.13	0.06	2.47
	7	1.43	0.63	0.08	0.04	2.18
	9	1.44	0.75	0.07	0.04	2.45
20 μmol/L	0	1.50	0.63	0.08	0.04	2.25
	3	1.51	0.73	0.12	0.05	2.41
	5	1.64	0.77	0.08	0.03	2.52
	7	1.38	0.75	0.21	0.11	2.45
	9	1.70	0.97	0.06	0.04	2.77

glycoside, and less aglucon exist in normal cell, baicalin is a state of baicalein stored.[39] Because additional hydroxyl can reduce the value of an electronic in phenol group, baicalein with dihydroxy have more anti-oxidant activity.[4,37,40] With the 0.002 μmol/L and 0.2 μmol/L H_2O_2, the expression of GUS was weaken [Figure 5], which may be that the finite H_2O_2 could be scavenged by existing flavones. Furthermore, because of aglucon synthesis prior to flavonoid glycoside, the GUS was a bit of redundant. At the third day, 20 μmol/L H_2O_2, the highest of all, coupled with poor efficiency of scavenger from the evidence that it did not up-regulated PAL and UBGAT more than that of the 0.002 μmol/L and 0.2 μmol/L [Figure 5], and that the CAT and PPO activities had been lower heavily, must brought about accumulation of H_2O_2. The highest amount of H_2O_2 has double effects. One is that too many H_2O_2, if not be scavenged in timely and converted into OH, maybe cause great damage, but this damage seems to can be relieved to some extent by the transformation of flavonoid glycoside into aglucon [Figure 4], and by special mechanism of conversion of wogonoside and wogonin into baicalin and baicalein with higher activities [Figure 3].[1] The other is messenger role. The accumulation of H_2O_2 procrastinated PAL expression about 3 days [Figure 5], allowing H_2O_2 act more time to strengthen induction. At the fifth day, the expression of both the PAL and the GUS were also enhanced remarkably, with the results of that baicalein increased from 0.19% to 0.57% and wogonin from 0.11% to 0.30% at the seventh day [Figure 5]. The following consequence is that anti-oxidant capacity was improved heavily through more aglucon, a process similar to adaption of *S. baicalensis* to semi-arid environment at natural condition.[1-3]

An obvious dose-response relationship between H_2O_2 concentrations and baicalein contents could be showed, the higher H_2O_2 concentration, the higher the baicalein contents, but the later it took action.

CONCLUSION

The exogenetic H_2O_2 damage the anti-oxidative enzyme system, but activate nonenzyme anti-oxidative system. The anti-oxidative enzyme system and nonenzyme system team up to scavenge excessive ROS. H_2O_2 is product of stresses consequence, the plant with treatment of H_2O_2 may be considered as a basic model of physiological and biochemical process in stresses. The produced flavones not only are critical to stress, but also essential materials in clinic. It elucidates the nature of herbal drug improvement, with brighter prospect to practical application. H_2O_2 is broken down into H_2O without any side effects; therefore, it is a good way to improve herb medicine quality.

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Conflicts of interest

There are no conflicts of interest.

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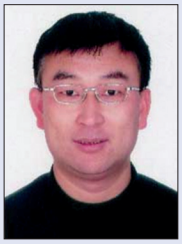

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